ASSESSING VERTICAL DISTRIBUTION OF ORGANIC CARBON STOCKS IN SHALLOW SOILS UNDER A BUSH-ENCROACHED RANGELAND

BY

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A MINI-DISSERTATION SUBMITTED IN (PARTIAL) FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE
IN
SOIL SCIENCE

FACULTY OF SCIENCE AND AGRICULTURE
(SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES)

AT THE

UNIVERSITY OF LIMPOPO, SOUTH AFRICA

SUPERVISOR: DR PE DLAMINI

2021

Declaration

I Abel Lesetja Masotla declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Soil Science) has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and execution, while related materials contained herein had been duly acknowledged.

Masotla AL (Mr)	Date

Dedication

I dedicate my report to God, my parents Mr. L.G and Mrs. M.J. Masotla, and my brothers Mr. M.J. Masotla, Mr. M.C. Masotla, and Mr. T.M. Ralekgokgo.

Acknowledgments

Firstly, I would like to thank almighty God for protecting and helping me throughout the study and for giving strength to complete this research. I would have not made it if it weren’t for Him and His favour upon me and for teaching me the value of hard work and dedication and their support in my studies, may God bless them at all times.

I acknowledge to parents Mr. L.G and Mrs. M.J. Masotla, family and friends Ms. M.N. Khumalo, Ms. R.E. Mashapa, Ms. ML Phooko, and Ms. M.R. Ntsewa for their love and support throughout the research work.

I would like to send gratitude and appreciation to my supervisor Dr. P.E. Dlamini and co-supervisor Mr. V.E. Mbanjwa for their patience, motivation, and support throughout the study; without their contributions to this study would have not been successful.

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Abstract

Globally and in most parts of South Africa, there is a trend of increasing shrub encroachment in savanna rangelands. A number of studies have investigated the impact of shrub encroachment on soil organic carbon content (SOC) and soil organic carbon stocks (SOCs) in savannas. So far there is no clear consensus on whether shrub encroachment increases or decreases the level of SOC and SOCs, especially in semi-arid savanna grasslands. Furthermore, knowledge on the effects of shrub encroachment on SOCs is largely restricted to the topsoil, as this is the part of the profile influenced by inputs and losses of soil organic matter. How shrub encroachment affects the vertical distribution of SOCs is rarely considered in the existing literature and the edaphic factors controlling SOCs with depth are poorly understood.

The objectives of this study were (i) to quantify the vertical distribution of SOC and SOCs and (ii) to identify the edaphic factors controlling the vertical distribution of SOC and SOCs in a shrub-encroached savanna grassland sited on shallow plinthic soil. To achieve the objectives, a vegetation and soil survey of the savanna grassland was conducted whereby sampling areas were demarcated and characterized into open and shrub-encroached grassland plots. In each encroachment level, three pits were randomly dug to the limiting layer on plots sited on the same soil type and similar topographic position. Soil samples were collected from the pits at depth intervals of 0-10, 10-20, 20-30, 30-40, 40-50, 50-60 and 60-70 cm. The collected soil samples were analyzed for chemical and physical properties in the laboratory. Correlation analysis was carried out to determine the relationship between SOC and SOCs, which were the variables of interest in this study and related controlling soil physicochemical properties.

The results showed that SOC was significantly greater ($P<0.05$) in the shrub-encroached grassland compared to open grassland. Furthermore, the results revealed that SOC was on average 19 and 13% greater in the topsoil (0-20 cm) and subsoil (20-70 cm) of shrub-encroached grassland compared to open grassland. The greater SOC in the topsoil of the shrub-encroached grassland was mainly attributed to higher SOC inputs from plant litter and detritus derived from trees and grasses, which are the dominant plant life forms in savannas. In the topsoil, SOC and SOCs were positively correlated with extractable phosphorus (P) ($r = 0.60$; $P < 0.05$), while in the subsoil they were positively correlated with extractable phosphorus (r = 0.54), soil porosity (r = 0.52), extractable copper (r = 0.46), extractable zinc (r = 0.41), exchangeable calcium (r = 0.37) and negatively correlated with mean weight diameter (r = -0.43). Overall, SOC and SOCs under both shrub-encroached and open grasslands vertically decreased with soil depth.

The results obtained highlighted that the factors controlling the level of SOC and SOCs differs in the topsoil and subsoil of the studied shrub encroached grassland. These findings suggest that in the shallow plinthic soil investigated in this study, SOC in the topsoil is controlled by the macronutrient P, while in the subsoil it is physically protected by soil aggregates and chemically stabilized via complexation interactions with exchangeable cations and heavy metals. In-depth understanding of the physico-chemical factors controlling SOC storage is critical to foster management practices that will improve the cycling of SOC in shrub-encroached savanna grasslands.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Shrub encroachment is the increase in biomass of indigenous woody plants (Jackson *et al*., 2002) or the invasion of destructive unwanted woody species causing an imbalance of the grass: shrub ratio, particularly in arid and semi-arid regions (Knapp *et al*., 2008). It is a natural phenomenon characterized by the excessive expansion of shrubs at the expense of herbaceous vegetation (Li *et al*., 2016; Hoffman and Jackson, 2000). The shift from previously open grasslands to shrub-encroached grasslands observed in savanna rangelands is a consequence of several factors such as overgrazing, fire suppression, soil moisture and nutrient availability (Archer *et al*., 1995; Van Auken, 2000). Wiegand *et al*. (2006) reported that soil moisture is one of the major limiting factor for grasses, as they use only topsoil moisture, while woody plants use moisture in subsoils. This implies that the balance between grass and bush production is determined by the relative availability of soil water and nutrients in different rooting depths (Dougill and Cox, 2007). Semi-arid grasses out-compete shrub species for soil moisture and nutrients in the topsoil layers, while woody species have a competitive advantage in the subsoils. This provides the opportunity for shrub species to increase and encroach into open grasslands (Britz and Ward, 2007). However, Ward (2005) argues that rooting depth cannot be used as an explanation for the initiation of shrub encroachment because young trees use the same subsurface soil layer as grasses in the early stages of growth. Several studies have moreover, reported that soil properties have contributed to a reduction in grass biomass production (Archer *et al*., 1995; Smit, 2005; Kgosikoma *et al*., 2012; O'Connor *et al*., 2014).

It is challenging to qualify an individual factor or a set of factors as the cause for shrub encroachment clearly because most factors are spatially related and scale-dependent, both over space and time (Scholes and Archer, 1997; Van Auken, 2000; Briske *et al*., 2003; Ward, 2005; Tessema *et al*., 2012; Belayneh and Tessema, 2017). Shrub encroachment directly affects the distribution of SOC and indirectly SOCs (Chiti *et al*., 2017). Meanwhile, SOC is the basis of soil fertility, as it plays an important role in soil biological, chemical and physical properties hence plant growth (Krull *et al*., 2003). Greater SOC helps in the maintenance of soil structure, nutrient cycling and other soil functions (Kulmatiski and Beard, 2013).

1.2 Problem statement

According to Ward (2005), approximately 10 to 20 million ha of rangelands in South Africa have experienced a decline in grazing capacity and biodiversity due to shrub encroachment. Shrub encroachment, which is common in savannas has recently surfaced as one of the top three recognized rangeland problems across 25% of the South African districts (O'Connor *et al*., 2014). The impacts of encroachment are envisaged to escalate over time depending on the natural resource management strategies applied in certain grassland areas (Hoffman *et al*., 1999; Higgins and Scheiter, 2013; O'Connor *et al*., 2014; Moncrieff *et al*., 2014). Some studies have reported that shrub encroachment is a serious problem in the North West, Northern Cape and Limpopo Provinces of South Africa. These studies further revealed that 42% of the rangelands in these provinces were already affected by shrub encroachment (Hoffman and Ashwell, 2001; Harmse, 2013). Furthermore, it has also been reported that shrub encroachment reduces water availability by up to 4%, and if the density of these encroaching woody species intensifies, reductions in water availability could expand to up to approximately 16% globally (O'Connor *et al*., 2014; Moncrieff *et al*., 2014). Consequently, shrub encroachment adversely affects available water resources, through a reduction in streamflow. Shrub encroachment also contributes to alterations of soil properties such as soil pH, SOC and nitrogen availability (Raich and Schlesinger, 1992; Wilcox, 2002).

1.3 Rationale

The effect of shrub encroachment on SOCs under semi-arid savanna grasslands has been widely reported in other areas worldwide (Archer, 1990; Bond and Midgley, 2000; Bond *et al*., 2003; Gibbens and Lenz, 2005; Ward, 2005). In the available literature, however, there is no clear consensus on the effect of shrub encroachment on SOCs, with many studies littered with conflicting and contradictory conclusions (Li *et al*., 2016). Some studies have reported that shrub encroachment increases SOCs (Podwojewski *et al.*, 2014; Jaleta *et al*., 2012). Others have reported that shrub encroachment has no impact on SOCs (Li *et al*., 2016), while other studies reported that encroachment results in a decrease in SOCs (Jackson *et al*., 2002; Qiu *et al*., 2012). Furthermore, the mechanisms of SOC stabilization and destabilization in grassland soils are also poorly understood (O'Brien and Jastrow 2013). Notably, most of these studies have focused on deep well-drained soils, with less attention accorded to shallow soils characterized by limiting layers. As shrub encroachment increases at an alarming rate, it is imperative that adequate attention is given to how this land cover transformation influences soil properties, especially SOCs which have been shown to be an indicator of soil degradation. The level of SOCs in grassland soils is driven by the interactions between SOC inputs via above-ground biomass accumulation and retention, as well as outputs primarily controlled by microbial decomposition of organic matter (Phillip *et al*., 1996). The decomposition and mineralization mechanisms that contribute to SOC storage in shallow soils limited by water availability is less known (Wiegand *et al*., 2005). It is apparent from published studies that the degree to which shrub encroachment affects the level of SOC and SOCs is not universally consistent (Smith and Johnson 2004; McKinley and Blair 2008). Thus, there is a need to investigate the effect of shrub encroachment on SOCs in shallow soils, and to identify controlling physico-chemical factors. In-depth understanding of the physico-chemical factors controlling SOC storage is critical to foster management practices that will improve the cycling of nutrients in shrub-encroached savanna grasslands.

1.4 Purpose of the study

1.4.1 The aim of the study is:

To investigate the effects of shrub encroachment on the vertical distribution of SOCs in shallow soil profiles of a shrub-encroached rangeland.

1.4.2 The objectives of the study are to:

i) Quantify the vertical distribution of SOC and SOCs in shallow shrub-encroached rangeland soil.
ii) Identify the physico-chemical properties controlling vertical distribution of SOC and SOCs in shrub-encroached rangeland soil.

1.4.3 Research questions

The project seeks to address the following research questions:

i) How does shrub encroachment affect the vertical distribution of SOCs in shallow shrub-encroached rangeland soils?
ii) Which physico-chemical properties have a major control on the level of SOCs under shrub-encroached rangeland soils?

1.5 Mini-dissertation structure

The mini-dissertation is arranged into four chapters, where the first chapter outlines the general introduction and provides a detailed description of the research problem and the aim of the study. Chapter 2 reviews and synthesizes existing literature on the impact of shrub encroachment on SOC and SOCs and also explains how edaphic factors control the vertical distribution of SOC. Chapter 3 provides information on how shrub encroachment affects the vertical distribution of SOC and SOCs, and isolates the edaphic factors that have a major control on the level of SOCs with soil depth in a shrub-encroached rangeland soil investigated in this study. In chapter 3, the results and discussion are explained and integrated to provide the significance of the research findings. The final chapter (chapter 5) summarizes and provides the overall conclusion of the study. Furthermore, the final chapter mentions recommendations for future research work.

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CHAPTER 2

LITERATURE REVIEW

2.1 Description of shrublands

Shrublands are a specific type of ecosystem in which shrubs are the dominant vegetation (Brand *et al*., 2009). Shrublands may either occur naturally or as a result of human activity. Natural occurrence of shrublands takes a long period through the succession process, whereby an open area is left unmoved and trees, grasses and shrubs start to grow. Shrublands that mainly results from human activities are mostly on old fields and pasture, and occur through mowing, abandoned agricultural lands or cutting of trees. There are multiple types of plant species that are present within shrublands, including grasses, shrubs and other herbaceous plants. Some shrublands are temporary because of the small shrubs that are easily burned by wildfires. Sometimes small shrubs are one stage in the development of shrub encroachment where larger shrubs will later grow up through them (Pariona, 2017). Shrublands provide important habitats for insects, fungi, birds and lizards. However, some shrub species are invading grasslands, and decreasing grassland productivity (Eldridge *et al*., 2011).

2.2 Definition of soil organic carbon content

Soil organic carbon is defined as the quantity of organic carbon that is stored in a given soil profile which has the capacity to either be accumulated or released in an ecosystem (Batjes, 1996). It can also be defined as the amount of organic carbon per unit mass area in a given soil (Dorji *et al.,* 2014). Soil organic carbon content determination should also be performed in a laboratory that has well established quality control and assurance systems (Throop *et al.,* 2012; Poeplau *et al.,* 2017; FAO, 2018). Accurate measurement of SOC assists in making the best decisions on land use and management practices that can improve soil health and productivity (Gross *et al.,* 2018). Furthermore, accurate estimation of SOC is also crucial for understanding the links between atmospheric and terrestrial organic carbon (Friedlingstein *et al.,* 2014).

2.3 Determination of soil organic carbon stocks

Quantification of SOCs depends upon accurate estimation of SOC concentration, bulk density and depth of a respective soil layer (Batjes, 1996; Poeplau *et al.,* 2017). The challenges encountered when calculating SOCs hinge on how to deal with the high spatial variability in SOC and properties associated with various soil and vegetation types, land use and management (Conant *et al.,* 2011; FAO, 2018). There are many studies that have reported various methods that can be used to calculate SOCs with different formulas and sampling strategies (Wang and Dalal, 2006; Poeplau and Don, 2013; Poeplau *et al.,* 2017; FAO, 2017). Soil sampling approaches or strategies can affect the variability of SOCs. The soil depth to which soil samples are collected for estimation of SOCs requires careful consideration. The characteristics of a single soil type can be defined on different scales using different sampling profiles which may lead to uncertainties in the estimation of SOCs (Zhi *et al.,* 2014). Poeplau *et al*. (2017) reported that some calculations produced systematically overestimated SOCs.

In the available literature, accurate calculation of SOCs depends on the use of parameters such as bulk density (BD) and the content of rock fragments (Wang and Dalal, 2006; Poeplau and Don, 2013; Poeplau *et al.,* 2017). For example, FAO (2017) used the following equation:

$$SOCs = \sum_{i=1}^{n} BD_i \times OC_i \times d_i \qquad \text{equation 1}$$

Where SOCs is soil organic carbon stocks of the investigated soil layer (kg C m^{-2}), BD is the bulk density (kg m^{-3}), OC is organic carbon content of soil (g C kg^{-1}) and d is the depth of sampled soil layer (m). It has been reported, however, that equation 1 leads to uncertainties on the calculated SOCs because it does not account for rock fragments (Schrumpf *et al.,* 2011). Rock fragments are sometimes not considered because coarse material in the soil can be very low (Dorji *et al*., 2014). In order to accurately quantify SOCs, the rock fragments should be accounted for during the calculation of BD as shown in equation 2 below. Where p(rock fragments) is the density of rock fragments.

$$\mathrm{BD}_{\text{fine soil}} = \frac{\text{mass}_{\text{sample}} - \text{mass}_{\text{rock fragments}}}{\text{volume}_{\text{sample}} - \frac{\text{mass}_{\text{rock fragments}}}{\rho_{\text{rock fragments}}}}, \qquad \text{equation 2}$$

The soil bulk density should be determined in the same core in which SOC is measured because the core method and the excavation method provide the most accurate determination of bulk density (FAO, 2018). However, it is not easy to adequately and accurately sample for bulk density in the field. When the effect of the spatial and temporal variation in bulk density is not totally corrected for, the estimates of SOCs to a fixed depth are mostly biased (Lee *et al*., 2009).

2.4 Stabilization and destabilization of SOC in shrub-encroached grasslands

Carbon can be stabilized in the soil by physical, chemical and biochemical mechanisms (Six *et al*., 2002; Six *et al*., 2006; Jastrow *et al*., 2007; Kane, 2015; FAO, 2017;). Physically, carbon may be stabilized via its encapsulation inside soil micro- and macro-aggregates where it is inaccessible to soil organisms. Chemically, carbon may be strongly adsorbed to clays via chemical bonds, which prevent its consumption by micro-organisms. Biochemically, carbon may be re-synthesized into complex molecular structures that may hinder decomposition (FAO, 2017). The organic matter with recalcitrant compounds takes a longer period to decompose (Fontaine *et al*., 2007).

The three mechanisms depend on a number of biotic, abiotic and management factors that shape their soil carbon stabilization efficacy (Six *et al*., 2006; Kane, 2015). There are various physical properties such as silt, clay content and micro-aggregation of the soil that are thought to be involved in the physical protection of soil organic matter from decomposition (Boutton *et al*., 2009). The mechanisms which are involved in the protection of soil organic matter from decay are: (i) biochemical recalcitrance inherited from original plant; (ii) physical protection by aggregates of biological and mineral particles held together by exudates, roots, and fungal hyphae; and (iii) physicochemical associations with silt and clay surfaces (Baldock *et al*., 2004; Huang *et al*., 2005). The accumulation of SOC is favoured by the formation of aggregates, which means that SOC is physically encapsulated from mineralization. Generally, the mineralization of SOC is more rapid in coarse-textured soil (Krull *et al*., 2003).

Previously, the stabilization mechanisms of SOC have been largely discussed with respect to their relationship between soil structure, aggregate formation, the ability of soil to stabilize SOC and its distribution within the soil profiles (Six *et al.,* 2002; Krull *et al.,* 2003). The accumulation and distribution of SOC emanates from stabilization and destabilization mechanisms that, in turn, are influenced by various factors such as chemical composition, allocation of plant inputs and factors that alter soil biota and aggregates (FAO, 2015). It has been reported that shrub-encroached grasslands have more plant litter (leaves and roots) that contribute to soil organic matter inputs into the soil (Montané *et al.*, 2010). Encroached woody plant species are the main sources of SOC through litter production and root exudates. The importance of woody plant root inputs for soil organic matter formation is attributed to both chemical composition and dead roots that increase aggregate formation and microbial carbon use efficiency (Six *et al.,* 2006).

Shrub encroachment into grasslands has a strong potential to modify SOC dynamics (Mureva *et al.,* 2018). A number of studies have shown that below-ground plant inputs contribute to organic matter, which is stabilized in the soil for long periods particularly in deep soil horizons (Kuzyakov and Domanski 2000; Rasse *et al*., 2005; Mendez-Millan *et al.,* 2010; Rumpel and Kögel-Knabner 2011; Clemmensen *et al.,* 2015; Dignac *et al.,* 2017). Below-ground inputs contribute to SOC storage through the tenacity of plant residues and via stimulation of soil microbial activity (Dignac *et al*., 2017). Above-ground litter inputs are transferred into the mineral soil where decomposition rates decrease with increasing depth (Prieto *et al*., 2016). Root litter contributes about one-third of the total litter inputs in grassland soils (Freschet *et al.,* 2013). Encroachment of woody plants has different effects in the soil depending on the type of woody plant species, thickness and depth (vertical distribution) of the plant roots (Freschet *et al*., 2013). The vertical distribution of SOC in the soil profile can change following a land cover transformation from open grassland to shrub-encroached grassland. In shrub-encroached grassland, the woody plant root biomass is comprised of large sized and lignified roots that are longer lived and less readily decomposed than grass roots, henceforth contribute less organic matter to the soil (Guo *et al*., 2018).

The stabilization of SOC plays an important role in grasslands by increasing the SOC stored within the soil (Wang *et al.,* 2015; Sarker *et al.,* 2018). The stabilization mechanisms of SOC is linked to physical protection by soil aggregates associated with the nature of recalcitrant compounds of SOC (Throckmorton *et al.,* 2015; Dignac *et al*., 2017). Physical protection plays a key role in the protection of SOC (Almendros and González-Vila, 1987; Ekschmitt *et al*., 2008; Kleber *et al*., 2011; Guo *et al*., 2018). The extent to which SOC is stored depends on the degree to which new organic matter inputs are stabilized and protected from decomposition (Boutton *et al.,* 2009).

2.5 Factors controlling soil organic carbon storage

The SOC available in the soil is the net balance between C inputs from plant production and losses through decomposition and leaching (Dorji *et al*., 2014). The distribution, storage and stability of SOC is influenced by various factors such as biotic factors (the abundance and vigour of soil faunal and microbial species), as well as environmental factors like temperature, moisture and soil texture (Lorenz and Lal, 2005). It has been reported that in topsoil's, SOC storage is mainly dependent on climatic variables, whereas SOC in the subsoils is controlled by soil texture. Soil texture plays a vital role through soil clay content which has a significant positive effect on SOC storage (Zhao *et al.,* 2017). When soil clay content increases, the fixation of soil organic matter is improved, and the decomposition of organic matter by microorganism decreases, ultimately leading to increased SOC levels (Gross and Harrison, 2018). This is because soils with higher clay content increase the potential for aggregate formation, which impede the process of SOC decomposition (Rice, 2002). Jobbágy and Jackson (2000), reported that soil texture is important to SOC dynamics, with subsurface SOC retention positively associated with clay content. However, uncertainty remains in the literature regarding the mechanisms controlling SOC and the factors that control SOC storage such as land use, vegetation cover intensity and parent material (Minasny *et al*., 2013).

A number of studies have shown that environmental factors such as temperature and precipitation control the vertical distribution and storage of SOC (FAO, 2017; Zhao *et al.,* 2017). Soil organic carbon content increases with increasing precipitation and decreasing temperature due to high decomposition of organic matter through increased microbial activity (Zak *et al*., 2000; Hobley *et al*., 2015). Generally, arid and semi-arid climate areas with higher temperatures have lower SOC levels and higher microbial organic matter decomposition than areas with lower temperatures (Ontl and Schulte, 2012). Temperature has been shown to have a stronger association with the vertical distribution of SOC than precipitation, because increased temperatures influence the SOC balance by limiting water availability (Wang *et al*., 2008). Badgery *et al*. (2014) reported that climate influences SOC storage based on different soil depths. This work indicated that near the soil surface (0-10 cm) climate is more influential on SOC, however at deeper depths (20-30 cm) soil texture and mineralogy become more influential to SOC storage.

Another factor is grazing, which tends to break soil aggregates apart and therefore increase SOC mineralization as broken aggregates are accompanied by changes in the chemical structure of SOC. Grazing history (grazing intensity, frequency and duration) causes changes in vegetation, soil physical and chemical properties through the selective feeding of plants and trampling of grasslands, which affects the formation process of soil aggregates (Sinsabaugh *et al*., 2013; Wang *et al*., 2020).

2.6 Effect of shrub encroachment on soil organic carbon content and stocks

It is apparent from many studies in the existing literature that the degree to which shrub encroachment affects the level of SOC and SOCs is not universally consistent (Smith and Johnson 2004; Montané *et al.*, 2007; McKinley and Blair 2008). There are conflicting results on the effects of encroachment on SOC and SOCs. For example, a study by Podwojewski *et al.* (2014) reported 69% decrease in SOC and 66% increase in SOCs at 0.2 m depth in a semi-arid area that received 745 mm mean annual precipitation (MAP). Similarly, Jaleta *et al*. (2012) and Jackson *et al*. (2002) reported more than 10% decrease in SOC at 0.1 m depth under similar climatic conditions. In contrast, other studies have reported more than 60% increase in SOC and decrease in SOCs at a depth of 0.6 m in a semi-arid climate with MAP ranging from 230-660 mm (Jackson *et al*., 2002; Smith and Johnson 2004; Li *et al*., 2016; Chiti *et al*., 2017). Li *et al*. (2016) reported that shrub encroachment had no impact on SOC and SOCs within the top 60 cm soil depth in a semi-arid region. Chiti *et al*. (2017) hypothesized that SOC levels increase during the transition from grassland to shrub-encroach grassland due to both the increase in carbon inputs to soil via litter deposition and the presence of herbaceous vegetation during natural succession. Scholes and Archer (1997) argued that the level of SOC increases because of the increase in the soil nutrient pool, especially in deeper soils. The increase of SOC in the subsoil under encroached areas could be also related to the increase in soil cover due to the increased number of trees that protect the soil from heavy rains and thus reduce the loss of SOC as dissolved organic carbon (Chiti *et al*., 2017). These contrasting results support the notion that SOC and SOCs can vary in shrub encroached grasslands depending on the geographic location, soil type, depth and climatic conditions of the investigated area (Powers *et al*., 2011).

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CHAPTER 3

ASSESSING VERTICAL DISTRIBUTION OF SOIL ORGANIC CARBON STOCKS IN SHALLOW PLINTHIC SOIL OF A SHRUB ENCROACHED RANGELAND IN LIMPOPO PROVINCE, SOUTH AFRICA

Abstract

Knowledge of the vertical distribution of soil organic carbon (SOC) content and stocks (SOCs) associated with land cover transformations caused by shrub encroachment is crucial because deep soil horizons have been shown to have greater capacity to sequester SOC. Despite this, deeper SOC dynamics remain poorly understood, particularly in plinthic soils under semi-arid savanna grasslands. Soil organic carbon stocks play a vital role in soil fertility in savanna grasslands, but the factors that control SOCs are not well-known. The estimates of SOC in the soil are characterized by large uncertainties due to poor understanding of the vertical distribution of SOC. The objective of this study was to quantify the vertical distribution of SOC and SOCs in shrub-encroached grassland compared to open grassland soil. In the topsoil, shrub-encroached grassland had lower SOCs compared to open grassland, while SOC was greater in shrub-encroached grassland in both the topsoil and subsoil (20-70 cm). SOCs of moderately acidic soil in shrub-encroached grassland was less compared to open grassland in the subsoil. For both topsoil and subsoil, SOC had positive correlations with SOCs ($r = 0.65$-0.66), porosity ($r = 0.52$-0.65: $P<0.05$) phosphorus ($r = 0.54$-0.60), polyvalent cations ($r = 0.37$-0.46); and SOCs had positive correlations with porosity ($r = 0.66$). The findings of this study suggest that SOCs and SOC is more chemically stabilized in the subsoil due to the interaction with polyvalent cations.

Keywords: plinthic soil, savanna grasslands, shrub encroachment, soil organic carbon, vertical distribution,

3.1 Introduction

Encroachment of bushes, shrubs and/or woody plants into previously open grasslands has been observed in many parts of the world. Studies have reported that shrub encroachment has a significant impact on grasslands, which occupy between 20-40% of the earth's land cover (Dixon *et al*., 2014; FAO, 2015;). Shrub encroachment is the increase of woody plant densities to an extent that the natural equilibrium of the woody plant (trees and shrubs) layer and herbaceous (grass and forb) layer densities is shifted unfavourably (Jobbágy and Jackson, 2000; Li *et al.,* 2016) and sometimes is accompanied by the degradation of grasslands. The encroachment by woody species is a form of degradation, with the extreme and undesirable increase in the abundance of woody plants resulting in the suppression of palatable grasses (Ward, 2005).

The shift from previously open grasslands to shrub-encroached grasslands, which is often irreversible, can result in various ecological consequences, such as changes in biodiversity (Li *et al*., 2016). Shrub encroachment also alters soil microbial community (Knapp *et al*., 2008), soil structure (Podwojewski *et al*., 2014), soil water content (Thomas *et al*., 2018), and influences carbon cycling in grassland ecosystems (Knapp *et al*., 2008; Throop and Archer, 2008; Li *et al*., 2019). Changes in vegetation cover can potentially influence soil organic matter, a key component for sustainable soil fertility and can also ultimately alter the SOC and SOCs dynamics in grasslands.

Soil is the largest terrestrial organic carbon reservoir in the biosphere, and stores almost three times the amount of carbon contained in plants and the atmosphere combined (Post *et al.,* 1982; Wang *et al.,* 2008; Dorji *et al.,* 2014; Gross and Harrison, 2018). Soil carbon storage is a key ecosystem service and SOCs play a vital role in soil fertility, but the factors that control these stocks are poorly understood (Manning *et al*., 2015). The estimates of SOC in the soil are characterized by large uncertainties due to poor understanding of the vertical distribution of SOC and stabilization and destabilization of SOC in both topsoils and subsoils (Jobbágy and Jackson, 2000).

The vertical distribution of SOC in relation to land cover transformations caused by shrub encroachment is crucial because deep soil horizons have been shown to have greater capacity to sequester SOC. Knowledge on the effects of shrub encroachment on SOCs in semi-arid grasslands is largely restricted to the topsoil (Ward *et al*., 2018; Dlamini *et* *al*., 2019), as this is the part of the soil profile influenced by inputs and losses of organic matter. How shrub encroachment affects subsoil SOCs is rarely considered and also factors controlling SOCs with depth are less well understood. Recent work by Dlamini *et al*. (2019) indicated that the mechanisms by which edaphic factors influence the stabilization and destabilization of SOCs shrub-encroached grasslands are still largely unknown. It is necessary to quantify the vertical distribution of SOCs and also to determine the stability of the SOC with depth under shrub-encroached plinthic soils. The objectives of this study were to quantify the vertical distribution of SOC and SOCs and intuit the physico-chemical properties controlling the vertical distribution of SOC and SOCs in shrub-encroached rangeland soils.

3.2 Methodology

3.2.1 Site description

The experimental site is located at the University of Limpopo experimental farm (Syferkuil) (29° 21'S; 29°41'E), which is situated near Mankweng in the Capricorn district in Limpopo Province, South Africa (Figure 1). The climate of the site is characterized by hot dry summers and cool dry conditions in winter, with average annual rainfall ranging between 400-600 mm per annum and average temperatures ranging between 4-27°C. The site elevation ranges between 1230-1237 meters above sea level (m.a.s.l). The soil is derived from a granitic parent material, characterized by a shallow Orthic A horizon (0-20 cm) underlain by a soft plinthic B horizon, classified as Westleigh soil form (Soil Classification Working Group, 1991) or Plinthic (IUSS Working Group WRB, 2015). The vegetation in the area is dominated by bushveld grasses such as *Eragrostis rigidior* and *Schmidtia pappophoroides,* while the woody component is dominated by Acacia species such as *Vachellia hebeclada and Opuntia ficus indica* (Mucina *et al*., 2006).

The study site is on grassland, which is characterized by patches of open grass grassland interspersed with occasional small shrubs and trees. For the past 30 years, the site has been used for livestock grazing wherein 60 cows per 20 ha [equating to 0.33 ha/LSU (hectare per large-stock unit)] are allowed to graze during summer months, with the site rested in winter. In recent times, it has been observed that there is a severe increase in tree density (number of trees per unit area) into previously open grassland (Mogashoa *et al.,* 2021).

3.2.2 Field sampling

A survey of the study site was conducted whereby areas that have less than 500 trees per hectare were identified and characterized as open grassland and areas greater than 1000 trees were considered as intensively shrub-encroached (Mogashoa *et al.,* 2021). From each encroachment level, three plots (10 x 10 m each) were randomly selected per encroachment level. In each plot, a soil pit was dug and the opened soil profile (A, B and C) was classified using the South African soil classification system (Soil Classification Working Group, 1991) then translated to the WRB soil classification system (IUSS Working Group WRB, 2015). Soil colour was determined using the Munsell colour chart. Bulk soil samples (1 kg) were collected down the soil profile at 10 cm depth intervals, up to the limiting layer (soft plinthites), a feature characteristic of shallow soils in this region. The collected soil samples from each soil profile were packed in plastic bags and transported to the laboratory at the University of Limpopo for further analyses. Once in the laboratory, the field moist samples were air-dried and sieved (<2 mm) for further soil physical and chemical analyses.

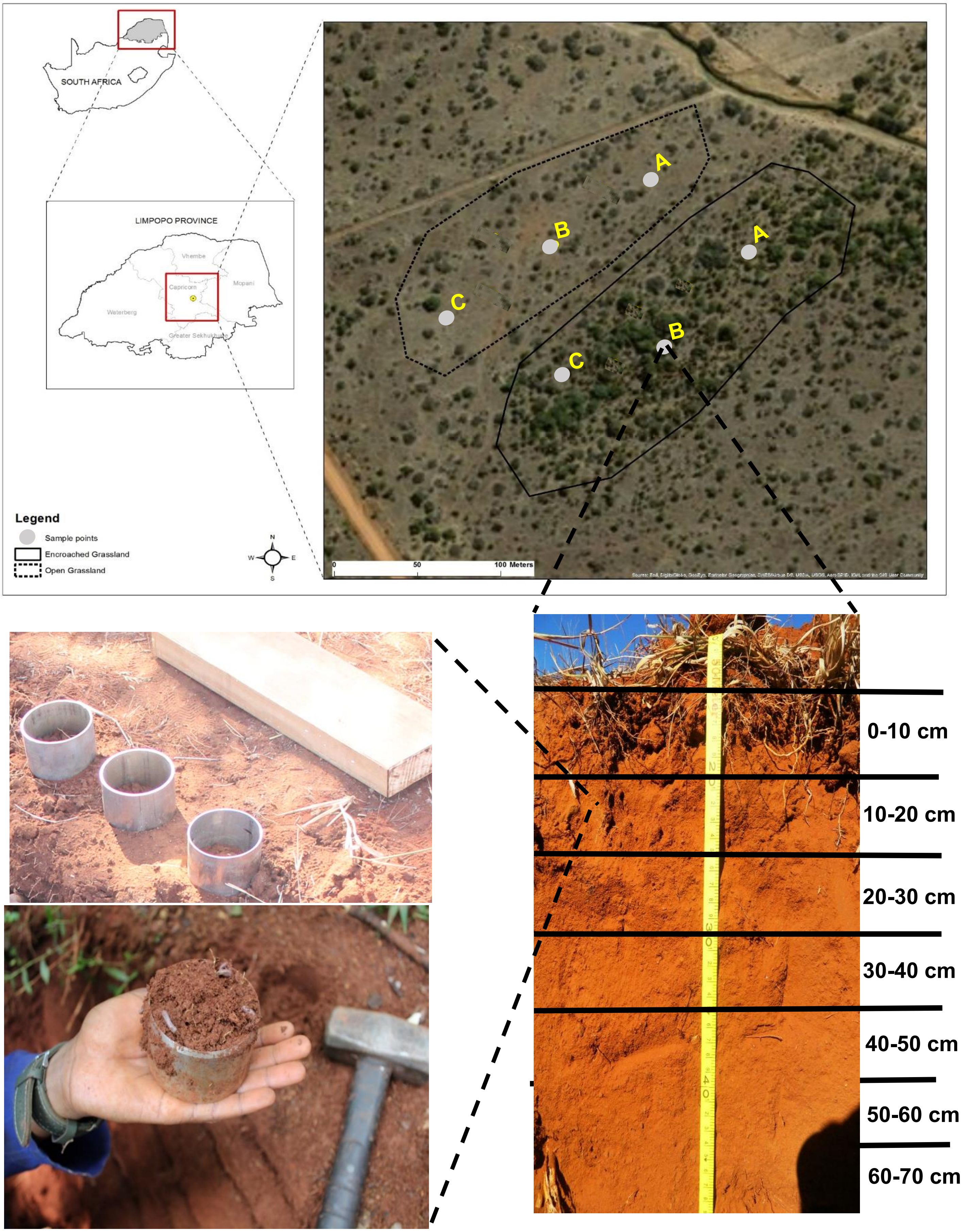

Figure 1: Location of the study site showing open and shrub-encroached grassland sampled points (A, B and C) and photograph showing the depth intervals where the soil samples were collected as well as an example of the metallic cylindrical cores used for collecting soil bulk density samples at the University of Limpopo's Syferkuil Farm.

3.2.3 Soil bulk density sampling

Soil bulk density was determined using the core method (Blake and Hartge, 1986). Additional three cores for bulk density, which is important to determine SOCs were collected from each depth interval. Soil bulk density was determined by taking undisturbed soil samples of known volume from each increment depth. In this study, a metallic cylindrical core of 0.078 m diameter and height of 5 cm was carefully hammered into the soil surface excavating the soil from around the cylinder and thereafter cut by a knife to remove the excess soil from the bottom of the cylinder. The undisturbed core samples were carefully stored in black crates for further analysis in the laboratory.

In the laboratory, core samples for bulk density were immediately oven-dried at 105^{o}C to determine the oven-dry weight. In this study, the bulk density of soil, which is the mass per unit volume was expressed as g cm^{-3}. Once the bulk density is known, measurements of soil mass, volume or percentages can be expressed in absolute terms (e.g. from g kg^{-1} soil to kg ha^{-1}). Bulk density was determined using the following equation:

$$BD = \frac{(W_2 - W_1)}{V}$$

Where W_2 is wet mass of the soil (g), W_1 is oven-dried mass of the soil (g) and V is the volume of the soil core (cm^3).

3.2.4 Particle size distribution and chemical soil properties

Particle size distribution of bulk soil samples were determined using the hydrometer method (Bouyoucos, 1962). Soil pH and electrical conductivity (EC) were determined using the Hanna Edge pH model HI763100 0-200 um/cm meter (Rhoades, 1982). Soil organic carbon was determined using the Walkley-Black method (Walkley and Black, 1934). The soil samples were also analyzed for exchangeable Ca, Mg (NH_4OAc), extractable acidity (1M KCl), P, K, Zn, Mn, and Cu (Ambic-2, an extractant containing 0.25 M NH_4HCO_3) (Manson and Roberts, 2000). Effective cation exchange capacity (ECEC) was calculated as the sum of KCl-extractable Ca, Mg, and acidity and Ambic-2 extractable K. Percentage acid saturation of the ECEC were calculated as ‘extractable acidity’ X 100/ (Ca + Mg + K + ‘extractable acidity’).

3.2.5 Determination of soil organic carbon stocks.

Soil organic carbon stocks in both open and shrub-encroached rangeland soil profiles were calculated using the equation below (FAO, 2017). In this study coarse fragments (plinthites) were not accounted for when calculating SOCs because coarse fragments content in the soil were generally low. Henceforth, the inclusion or exclusion of coarse fragments is not expected to significantly alter the calculation.

$$SOCs = \sum_{i=1}^{n} BD_i \times OC_i \times d_i$$

Where SOCs is soil organic carbon stocks (kg C m^{-2}), BD is the bulk density (kg m^{-3}), OC is organic carbon (g C kg^{-1}) and d is the depth interval thickness of the sampled soil layer (m).

3.3 Statistical analysis

Basic statistics of the data including minimum, maximum, average, median, standard deviation and coefficient of variation were computed following procedures by Webster (2001). A two-way analysis of variance (ANOVA) was run to test the effect of encroachment level and depth on SOC, BD and SOCs using GenStat. To identify the physico-chemical factors controlling SOC and SOCs, Pearson’s correlation coefficient (r) was used to test the strength of the correlation between SOC, BD and SOCs and soil properties using STATISTICA 7.0.

3.4 Results

3.4.1 Morphological and physico-chemical characteristics of soil

Table 1 shows the morphological and physico-chemical characteristics of soil in open and shrub-encroached grassland sampled sites. In profile A, the topsoil (0-20 cm) under shrub-encroached grassland was characterized by strong brown (7.5YR5/8) dry colour; loamy and sandy loam textural class, specifically with a particle size distribution of 18% clay, 30% silt, 52% sand, and a moderately acidic pH (KCl) ranging between 4.50 and 4.60. The subsoil (20-70 cm) is dominated by reddish brown (5YR5/4) dry colour; with a loamy texture characterized by a particle size distribution of 22% clay, 34% silt, 44% sand, and a slightly acidic pH (KCl) ranging from 4.46 to 5.18. The profile as a whole had an effective rooting depth of 70 cm. In open grassland, the topsoil was dominated by reddish brown (2.5YR5/4) dry colour; characterized by loamy sand (8% clay, 20% silt, 72% sand), and a moderately acidic pH (KCl) ranging between 4.27 and 4.77. The subsoil was characterized by strong brown (7.5YR5/8) dry colour with a loamy sand (10% clay,16% silt, 74% sandy), and a slightly acidic pH (KCl) ranging between 4.20 and 5.34.

Under shrub-encroached grassland in the topsoil, profile B was dominated by yellowish red (5YR5/8) dry colour; characterized by loamy texture (20% clay, 32% silt, 48% sand), while under open grassland, profile B was dominated by strong brown (7.5YR5/8) dry colour, characterized by sandy loam texture (16% clay, 28% silt, 56% sand). Under shrub-encroached grassland, the topsoil was moderately acidic with a pH (KCl) ranging between 3.96 and 4.2 and under open grassland the topsoil was also moderately acidic pH (KCl) ranging between 4.08 and 5.20. The subsoils under shrub-encroached and open grassland were dominated by strong brown (7.5YR5/8) dry colour, and yellowish red (5YR5/8) dry colour, characterized by sandy clay loam (28% clay,24% silt, 48% sandy) and sandy loam texture (13% clay, 27% silt, 60% sand) respectively. The pH (KCl) under both shrub-encroached and open grassland was slightly acidic, ranging between from 3.80 to 5.54.

From Table 1 in profile C under shrub-encroached and open grasslands, the soil colour was yellowish red (5YR5/8) in the topsoil. The textural class in the topsoil for both grass cover was distinguished by sandy loam (16% clay, 28% silt, 56% sand) and loamy sand (4% clay, 16% silt, 80% sand) with and a moderately acidic pH (KCl) ranging between 3.86 and 4.47. In the subsoil, profile C had brown (7.5YR5/8) and yellowish brown (5YR4/6) dry colour under shrub-encroached and open grasslands, respectively. Under both grass cover, the textural class was dominated by loamy sand (8% clay,20% silt, 72% sandy) and sandy loam (15% clay, 28% silt, 57% sand). The pH (KCl) of profile C ranged between 3.98 and 5.48, indicating that in the subsoil for both shrub-encroached and open grassland the soil was slightly acidic.

Table 1: Pedological and morphological characteristics of plinthic soil from open and shrub-encroached grassland.

Profile	Enchroach-level	Depth	pH (KCl)	Colour		%clay	%silt	%sand	Textural class
A	Encroached	0-10	4.59	7.5YR5/8	Strong brown	16	24	60	sandy loam
A	Encroached	10-20	4.6	7.5YR5/8	Strong brown	20	36	44	loam
A	Encroached	20-30	4.46	2,5YR4/8	Red	24	36	40	loam
A	Encroached	30-40	4.4	7.5YR5/8	Strong brown	20	32	48	loam
A	Encroached	40-50	4.85	7.5YR5/8	Strong brown	8	24	68	sandy loam
A	Encroached	50-60	4.97	7.5YR5/8	Strong brown	16	32	52	sandy loam
A	Encroached	60-70	5.18	5YR5/4	Reddish brown	12	28	60	sandy loam
B	Encroached	0-10	4.29	7.5YR4/8	Strong brown	16	28	56	sandy loam
B	Encroached	10-20	3.9	5YR5/8	Yellowish red	20	32	48	loam
B	Encroached	20-30	3.96	7.5YR5/8	Strong brown	12	24	64	sandy loam
B	Encroached	30-40	4.31	7.5YR5/8	Strong brown	28	24	48	sandy clay loam
B	Encroached	40-50	4.79	7.5YR5/8	Strong brown	20	32	48	loam
B	Encroached	50-60	5.27	5YR4/6	Yellowish red	16	32	52	sandy loam
B	Encroached	60-70	5.51	7.5YR5/8	Strong brown	12	24	64	sandy loam
C	Encroached	0-10	3.88	5YR5/8	Yellowish red	16	28	56	sandy loam
C	Encroached	10-20	3.86	5YR6/6	Reddish yellow	4	16	80	loamy sand
C	Encroached	20-30	3.98	7.5YR5/8	Strong brown	8	20	72	loamy sand
C	Encroached	30-40	4.52	5YR6/8	Reddish brown	8	20	72	loamy sand
C	Encroached	40-50	4.79	7.5YR5/8	Strong brown	12	28	60	sandy loam
C	Encroached	50-60	5.23	7.5YR5/8	Strong brown	16	28	56	sandy loam
C	Encroached	60-70	5.48	5YR5/6	Yellowish red	16	28	56	sandy loam
A	Open	0-10	4.775	5YR6/8	Reddish yellow	8	20	72	loamy sand
A	Open	10-20	4.275	2.5YR5/4	Reddish brown	16	24	60	sandy loam
A	Open	20-30	4.21	7.5YR5/8	Strong brown	8	16	76	loamy sand
A	Open	30-40	4.27	7.5YR5/8	Strong brown	12	16	72	loamy sand
A	Open	40-50	4.63	7.5YR5/8	Strong brown	24	32	44	loam
A	Open	50-60	4.775	7.5YR5/8	Strong brown	20	28	52	sandy clay loam
A	Open	60-70	5.34	5YR6/8	Reddish yellow	16	28	56	sandy loam
B	Open	0-10	5.2	7.5YR5/8	Strong brown	20	32	48	loam
B	Open	10-20	4.08	7.5YR5/8	Strong brown	16	16	68	sandy loam
B	Open	20-30	3.87	7.5YR5/8	Strong brown	16	24	60	sandy loam
B	Open	30-40	3.81	2.5YR5/8	Red	12	12	76	loamy sand
B	Open	40-50	3.96	5YR5/6	Yellowish red	24	32	44	loam
B	Open	50-60	4.54	5YR4/6	Yellowish red	8	16	76	loamy sand
B	Open	60-70	5.51	5YR5/8	Yellowish red	8	12	80	loamy sand
C	Open	0-10	4.35	5YR4/6	Yellowish brown	16	16	68	sandy loam
C	Open	10-20	4.47	7.5YR5/8	Strong brown	4	12	84	loamy sand
C	Open	20-30	4.55	7/5YR5/8	Strong brown	20	28	52	sandy loam clay
C	Open	30-40	4.73	7.5YR5/8	Strong brown	12	12	76	loamy sand
C	Open	40-50	5.3	7.5YR5/8	Strong brown	8	16	76	loamy sand
C	Open	50-60	5.01	5YR4/6	Yellowish brown	16	28	56	sandy loam
C	Open	60-70	5.17	5YR4/4	Reddish brown	12	16	72	loamy sand

3.4.3. Vertical distribution of SOC, BD and SOCs in open and shrub-encroached grassland

Table 2 shows the analysis of variance (ANOVA) of the effect of soil depth on SOC, BD and SOCs from open and shrub-encroached grassland. ANOVA analysis revealed that SOC was significantly greater in the shrub-encroached compared to the open grassland at all soil depths. Conversely, soil BD was significantly higher in the open grassland compared to shrub-encroached grassland, while there were no significant differences in SOCs in open and shrub-encroached grassland (Table 2).

For profile A, SOC was higher in the shrub-encroached grassland compared to the open grassland. Notably and as could be expected, the distribution of SOC in this profile declined with depth in both open and shrub-encroached grassland. In the shrub-encroached grassland, SOC in the topsoil (0-10 cm) decreased from 11.60 g kg^{-1} compared to 9.49 g kg^{-1} in the open grassland. A similar pattern was observed in the 10-20 cm soil layer, where SOC also decreased from 10.03 g kg^{-1} to 7.51 g kg^{-1} (Figure 2). Furthermore, SOC in both shrub-encroached grassland and open grassland increased at 20-30 cm depth. Under shrub-encroached grassland, SOC increased with depth from 9.49 g kg^{-1} to 10.24 g kg^{-1} and under open grassland SOC increased from 7.51 g kg^{-1} to 10.07 g kg^{-1}. In the 30-60 cm soil layer, under shrub-encroached grassland SOC further decreased with depth to 7.81 g kg^{-1} and then increased to 10.23 g kg^{-1} at 70 cm. Under open grassland a different trend was observed, where SOC decreased gradually with depth from 30 cm (10.07 g kg $^{-1}$) up to the 70 cm soil layer (8.90 g kg^{-1}).

In profile B, shrub-encroached grassland had more SOC compared to open grassland at the soil surface (0-10 cm). SOC decreased with depth (10-20 cm) under shrub-encroached grassland from 15.9 g C kg^{-1} to 9.49 g C kg^{-1}, where under open grassland SOC slightly increased from 10.94 g C kg^{-1} to 11.60 g C kg^{-1} in this soil layer. Less SOC was observed under shrub-encroached grassland in the subsoil, decreasing slightly from 10.71 g C kg^{-1} in the 20-30 cm layer to 10.15 g C kg^{-1} in the 60-70 cm soil layer, while open grassland had more SOC in the subsoil which increased from 10.35 g C kg^{-1} in the 10-30 cm soil layer to 11.04 g C kg^{-1} in the 60-70 cm soil layer (Figure 2).

In profile C, more SOC (14.83 g C kg^{-1}) was observed under shrub-encroached grassland in the soil surface (0-10 cm), decreasing to 10.37 g C kg^{-1} in the 10-20 cm soil layer, while under open grassland SOC slightly increased from 8.90 to 9.22 g C kg^{-1}. SOC then decreased with depth for both shrub-encroached grassland and open grassland in the subsoil. Under shrub-encroached grassland SOC increased in the 20-30 cm soil layer to 12.24 g C kg^{-1}, then gradually decreased up to the 60-70 cm soil layer (11.57 g C kg^{-1}), while under open grassland SOC decreased from 8.26 g C kg^{-1} in the 20-30 cm soil layer until the 60-70 cm soil layer (6.65 g C kg^{-1}).

Table 2: ANOVA of SOC, BD and SOC from open and shrub-encroached grassland at 95% confidence level.

SOC					
Encroach-Level	d.f.	s.s.	m.s.	v.r.	F pr.
Level	1	22.38	22.38	11.5	0.002
Soil Depth	6	40.57	6.76	3.47	0.011
Level.Depth	6	13.40	2.23	1.15	0.361
Residual	28	54.49	1.95		
Total	41	130.84			
BD					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Level	1	0.58	0.58	190.91	<.001
Soil Depth	6	0.01	0.00	0.71	0.642
Level.Depth	6	0.13	0.02	6.85	<.001
Residual	28	0.09	0.00		
Total	41	0.81			
SOCs					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Encroach-Level	1	0.05	0.05	1.61	0.215
Soil Depth	6	0.79	0.13	3.92	0.006
Level.Depth	6	0.15	0.03	0.76	0.605
Residual	28	0.94	0.03		
Total	41	1.93			

d.f: degrees of freedom; s.s: sum of squares; m.s: mean square; v.r: variance; F pr: p value

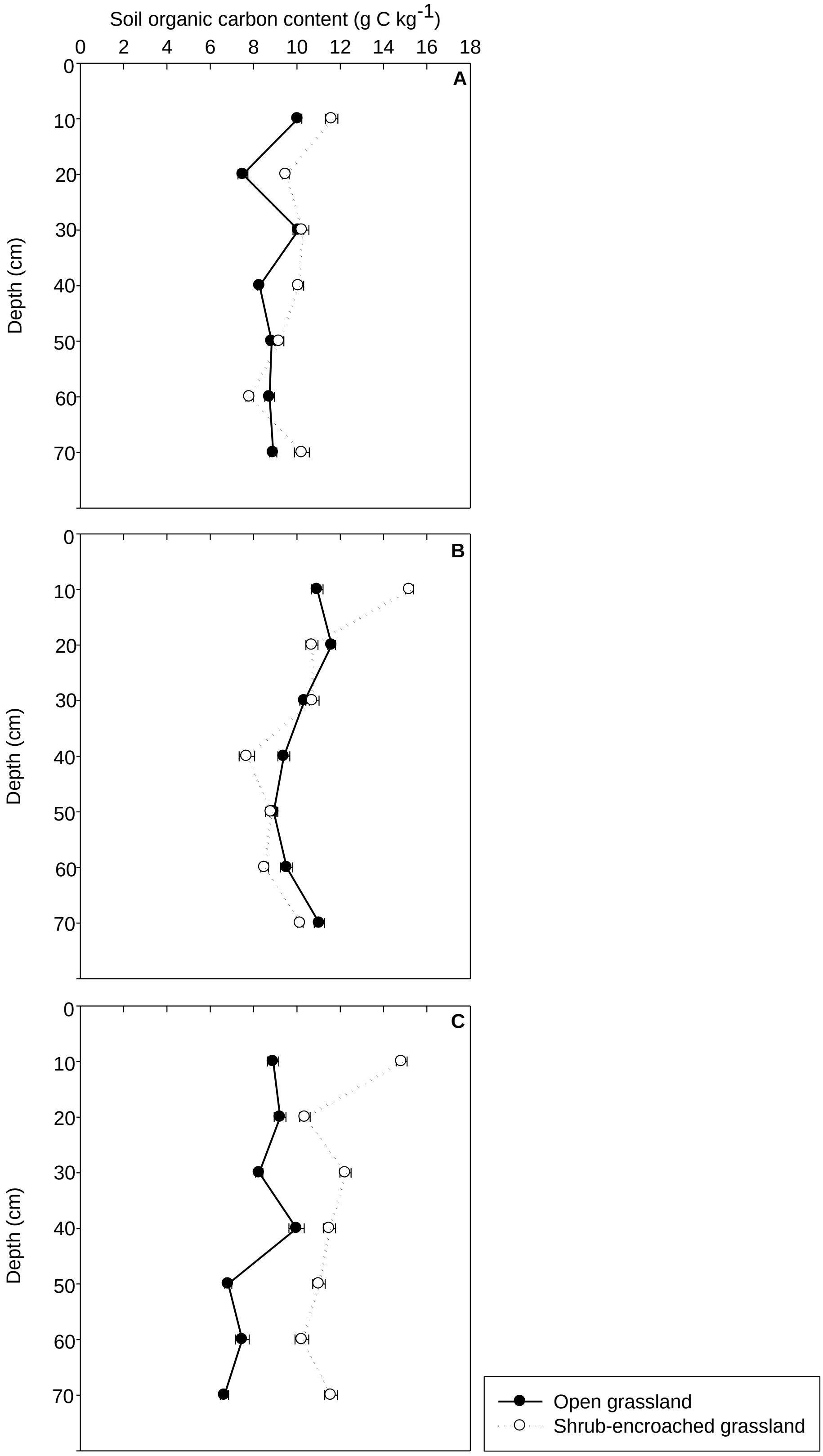

Figure 2: Vertical distribution of mean soil organic carbon (SOC) content in profiles A, B and C dug in the open and shrub-encroached grassland. The error bars represent standard error of the mean (n =3).

The soil bulk density (BD) in profile A under shrub-encroached grassland is lower compared to open grassland as shown in Figure 3. Under shrub-encroached grassland BD increased from the soil surface (0-10 cm) from 1.18 to 1.33 g cm^{-3} in the 10-20 cm soil layer. In the topsoil, different patterns were observed under open grassland where BD decreased in the 0-10 cm soil layer (1.56 g cm^{-3}) to 1.49 g cm $^{-3}$ in the 10-20 cm soil layer. Under shrub-encroached grassland, BD decreased up to 40 cm depth (1.29 g cm^{-3}), then slightly increased to 1.35 g cm^{-3} in the 40-50 cm soil layer and decreased further to 1.12 g cm^{-3} in the 60-70 cm soil layer. Under open grassland BD increased from 1.29 g cm^{-3} in the 30-40 cm soil layer to 1.44 g cm^{-3} in the 60-70 cm soil layer. For both open grassland and shrub-encroached grassland, the BD values decreased with depth.

In profile B from Figure 3, lower BD values were observed under shrub-encroached grassland as compared to open grassland. Under shrub-encroached grassland BD values increased from the 0-10 cm soil layer (1.24 g cm^{-3}) to 1.34 g cm^{-3} in the 10-20 cm soil layer, while under open grassland BD values slightly decreased from the 0-10 cm soil layer (1.50 g in cm^{-3}) to 1.46 g cm^{-3} in the 10-20 cm soil layer. In the subsoil, soil bulk density under shrub-encroached grassland decreased with depth from 10-20 cm (1.34 g cm^{-3}) up to 70 cm depth (1.10 g cm^{-3}), while open grassland showed a different trend where BD values increased from 1.45 g cm^{-3} at 20-30 cm soil depth up to 70 cm depth (1.60 g cm^{-3}).

In profile C, the topsoil under shrub-encroached grassland had lower BD values when compared to open grassland. Soil BD in the topsoil under shrub-encroached grassland increased from the 0-10 cm soil layer (1.04 g cm^{-3}) to 1.27 g cm^{-3} at the 10-20 cm soil layer, while under open grassland BD values decreased from the soil surface (1.49 g cm^{-3}) to 1.43 g cm^{-3} at 10-20 cm soil depth. In the subsoil under shrub-encroached grassland, BD increased with depth, from the 20-30 cm soil layer (1.25 g cm^{-3}) up to the 50-60 cm soil layer (1.32 g cm^{-3}) and then decreased to 1.17 g cm^{-3} at 60-70 cm soil depth. In profile C, open grassland showed a different pattern where BD slightly decreased from 1.43 g cm^{-3} (10-20 cm soil layer) to 1.35 g cm^{-3} at 20-30 cm soil depth, then increased up to the 60-70 cm soil layer (1.65 g cm^{-3}).

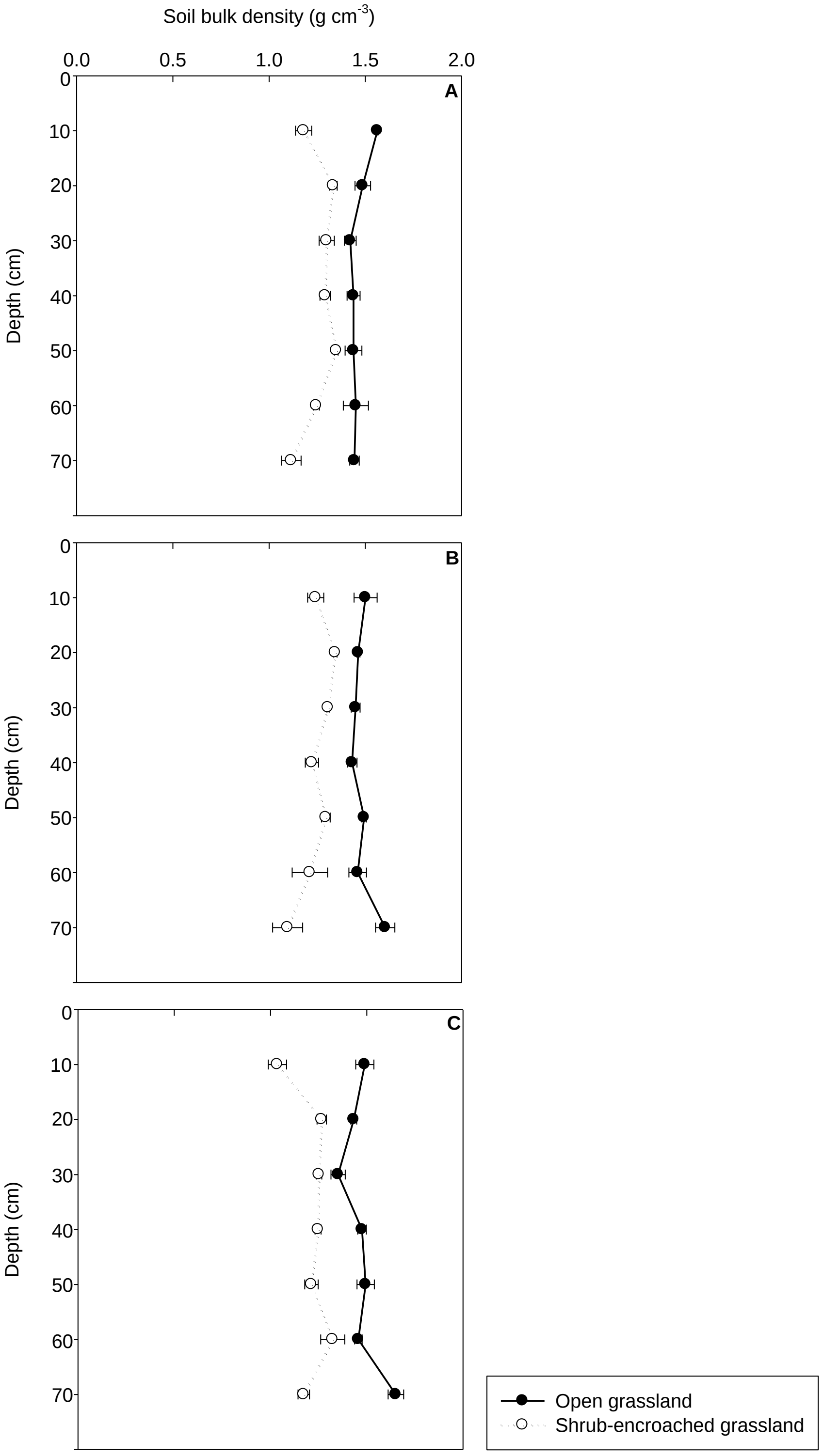

Figure 3: Vertical distribution of mean soil bulk density in profiles A, B and C dug in the open and shrub-encroached grassland. The error bars represent standard error of the mean (n =3).

From Figure 4, profile A shows that shrub-encroached grassland had less SOCs in the topsoil compared to open grassland. However, the SOCs under both shrub-encroached grassland and open grassland decrease with depth. Under shrub-encroached grassland, SOCs decreased from the soil surface (1.36 kg C m^{-2}) to 1.26 kg C m^{-2} in the 10-20 cm soil layer. A similar trend was also observed under open grassland where SOCs had a greater decrease from the soil surface (1.78 kg C m^{-2}) to 1.16 kg C m^{-2} in the 10-20 cm soil layer. Figure 4 showed that, in the subsoil SOCs under shrub-encroached grassland increased from 1.16 kg C m^{-2} in the 10-20 cm soil layer to 1.35 kg C m^{-2} in the 20-30 cm soil layer, then decreased up to 60 cm (0.98 kg C m^{-2}) and increased again to 1.11 kg C m^{-2} in the 60-70 cm soil layer. Under open grassland, SOCs increased from 1.16 kg C m^{-2} (10-20 cm soil layer) to 1.34 kg C m^{-2} (20-30 cm soil layer) then decreased in the 50-60 cm soil layer to 1.03 kg C m^{-3} and increased again to 1.42 kg C m^{-2} in the 60-70 cm soil layer.

In profile B (Figure 4), under shrub-encroached grassland there was a decrease in SOCs where shrub-encroached grassland had more stocks as compared to open grassland in the 0-10 cm soil layer. SOCs under shrub-encroached grassland decreased from 1.88 kg C m^{-2} in the 0-10 cm soil layer to 1.43 kg C m^{-2} in the 10-20 cm soil layer, while under open grassland SOCs had no change from the soil surface up to 20 cm soil depth (1.65 kg C m^{-2}). In the subsoil, SOCs under shrub-encroached grassland decreased with depth from 1.40 kg C m^{-2} (10-20 cm soil layer) up to the 60-70 cm soil layer (1.09 kg C m^{-2}). Under open grassland a different trend was observed, from which SOCs decreased from the 10-20 cm soil layer (1.65 kg C m^{-2}) up to the 50-60 cm soil layer (1.26 kg C m^{-2}), then increased again to 1.67 kg C m^{-2} in the 60-70 cm soil layer.

In profile C, shrub-encroached grassland was characterized by decreasing SOCs from the soil surface where shrub-encroached grassland had greater SOCs as compared to open grassland. Shrub-encroached grassland had 1.54 kg C m^{-2} at the soil surface, which then decreased to 1.31 kg C m^{-2} in the 10-20 cm soil layer. When compared to open grassland, SOCs increased from 1.35 kg C m^{-2} to 1.48 kg C m^{-2}. In the subsoil, SOCs for both shrub-encroached grassland and open grassland decreased with soil depth, where shrub-encroached grassland had higher SOCs values in the 60-70 cm soil layer (1.30 kg C m^{-2}) as compared to open grassland (1.10 kg C m^{-2}), shown in Figure 4.

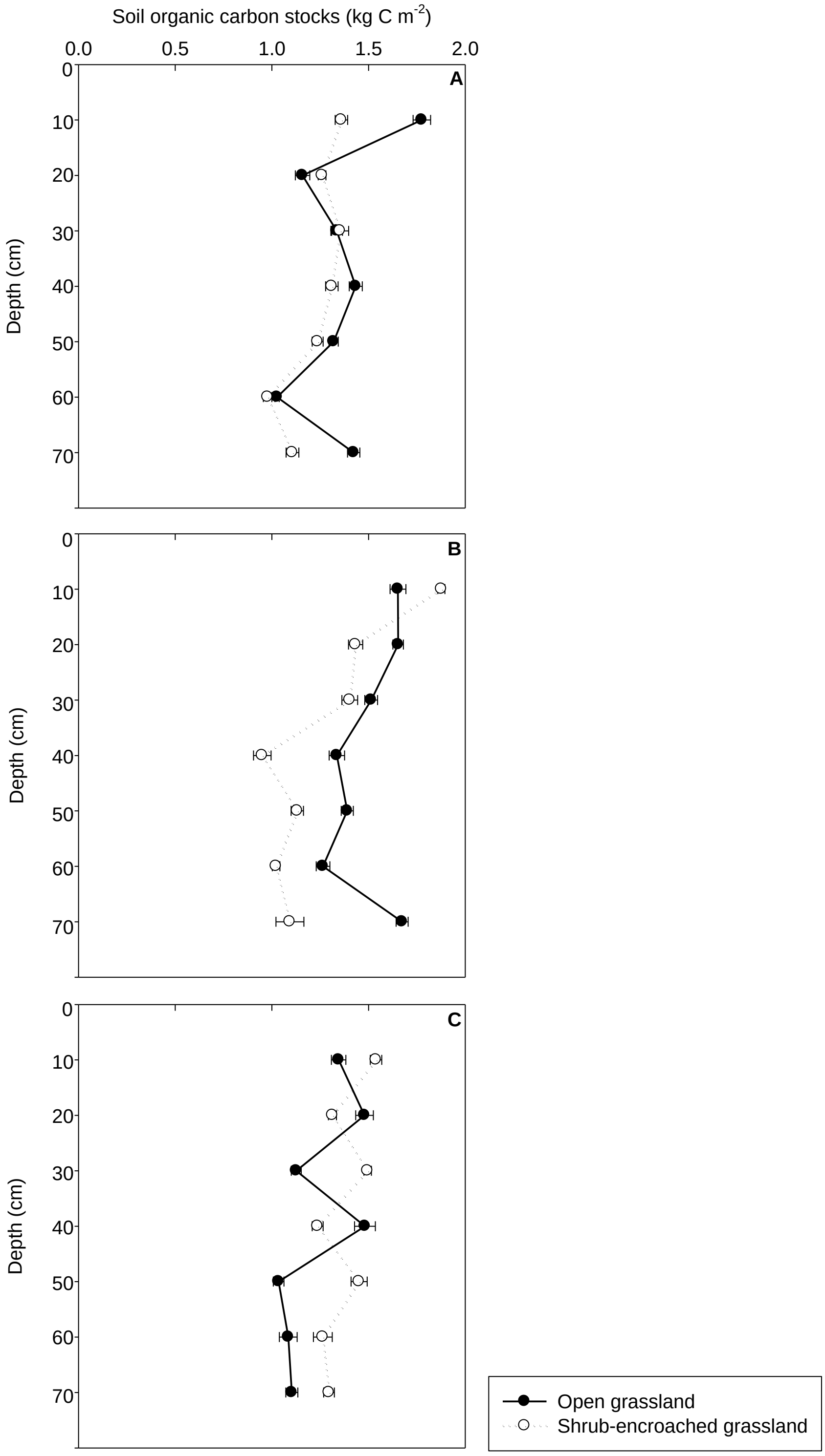

Figure 4: Vertical distribution of mean soil organic carbon stocks (SOCs) dug in the open and shrub-encroached grassland. The error bars represented standard error of the mean (n = 3).

3.4.2 Relationship between SOC, SOCs and selected soil properties in the topsoil (0-20 cm) and subsoil (20-70 cm) layers

Table 3 shows correlation between SOC, SOCs and selected soil properties in the topsoil and subsoil of shrub-encroached grassland compared to an adjacent open grassland. The SOC in the topsoil was positively correlated with SOCs (r = 0.65: $P < 0.05$), phosphorus (r = 0.60) and negatively correlated to soil bulk density (r = -0.70). In the subsoil, SOC was positively correlated with SOCs (r = 0.63: $P < 0.05$), porosity (r = 0.52), phosphorus (r = 0.54), calcium (r = 0.37), zinc (r = 0.41), copper (r = 0.46) and negatively correlated to soil bulk density (r = -0.40) and mean weight diameter (MWD) (r = -0.43).

As shown in Table 3 in the topsoil, SOCs were only positively correlated with Por (r = 0.66). In the subsoil, SOCs were positively correlated with more soil properties including exchangeable acidity (r = 0.43), acid saturation percentage (r = 0.38), manganese (r = 0.37) and negatively correlated with magnesium (r = -0.38).

Table 3: Correlation matrix showing Pearson's correlation coefficients between SOC, SOCs and selected soil properties in topsoil (0-20 cm) and subsoil (20-70 cm) of open and shrub-encroached grassland soil

Topsoil	BD	SOC	SOCs	Por	pH	MWD	clay	silt	sand	P	K	Ca	Mg	EA	ECEC	Acid-sat	Zn	Mn	Cu
BD	1.00																		
SOC	**-0.70**	1.00																	
SOCs	0.06	**0.65**	1.00																
Por	0.05	0.49	**0.66**	1.00															
pH	0.49	-0.24	0.22	0.19	1.00														
MWD	0.37	-0.16	0.12	0.36	-0.07	1.00													
clay	-0.09	0.18	-0.04	0.21	0.15	-0.50	1.00												
silt	-0.29	0.27	0.00	0.32	0.21	-0.55	**0.77**	1.00											
sand	0.22	-0.25	0.02	-0.29	-0.19	0.56	**-0.92**	**-0.96**	1.00										
P	-0.21	**0.60**	0.47	**0.58**	-0.43	0.28	0.17	0.05	-0.11	1.00									
K	-0.13	-0.25	-0.44	**-0.80**	0.04	-0.48	0.03	-0.01	-0.01	-0.38	1.00								
Ca	0.26	0.22	0.55	**0.85**	0.39	0.17	0.29	0.41	-0.38	0.23	**-0.80**	1.00							
Mg	0.38	0.04	0.47	**0.66**	**0.80**	0.22	0.05	0.17	-0.13	0.02	-0.47	**0.70**	1.00						
EA	-0.48	0.31	-0.11	0.16	**-0.80**	0.28	-0.22	-0.10	0.16	0.42	-0.44	0.03	-0.45	1.00					
ECEC	0.27	0.22	0.55	**0.85**	0.46	0.18	0.27	0.42	-0.38	0.21	**-0.77**	**0.99**	**0.77**	-0.03	1.00				
Acid-sat	-0.48	0.29	-0.13	0.11	**-0.80**	0.30	-0.24	-0.15	0.20	0.39	-0.39	-0.04	-0.49	**0.99**	-0.09	1.00			
Zn	-0.08	0.42	0.36	0.38	-0.45	0.23	0.12	-0.05	-0.02	**0.92**	-0.16	0.01	-0.09	0.23	-0.01	0.20	1.00		
Mn	-0.17	0.44	0.29	0.39	**-0.61**	0.24	0.11	-0.08	0.00	**0.94**	-0.30	0.03	-0.18	0.45	0.00	0.43	**0.96**	1.00	
Cu	-0.19	0.39	0.36	**0.76**	-0.06	0.35	-0.08	0.25	-0.12	0.26	**-0.85**	**0.75**	0.38	0.56	**0.73**	0.51	-0.02	0.11	1.00
Subsoil																			
BD	1.00																		
SOC	**-0.40**	1.00																	
SOCs	0.31	**0.63**	1.00																
Por	-0.27	**0.52**	0.29	1.00															
pH	-0.11	-0.16	-0.32	-0.09	1.00														
MWD	**0.56**	**-0.43**	-0.08	-0.18	0.33	1.00													
clay	-0.12	-0.30	-0.27	0.03	-0.15	-0.04	1.00												
silt	**-0.47**	-0.05	-0.31	-0.01	0.10	-0.34	**0.70**	1.00											
sand	0.34	0.17	0.32	0.00	0.01	0.23	**-0.90**	**-0.94**	1.00										
P	**-0.47**	**0.54**	0.18	**0.78**	0.30	-0.15	-0.07	0.01	0.03	1.00									
K	-0.03	0.03	0.11	-0.35	-0.28	**-0.38**	0.21	0.25	-0.25	**-0.37**	1.00								
Ca	-0.23	**0.37**	0.11	**0.71**	**0.52**	0.03	-0.13	0.00	0.07	**0.81**	**-0.58**	1.00							
Mg	0.09	-0.33	**-0.38**	**-0.41**	**0.86**	**0.45**	-0.25	-0.04	0.15	-0.08	**-0.38**	0.25	1.00						
EA	0.27	0.14	**0.43**	0.20	**-0.78**	-0.15	-0.10	-0.34	0.25	-0.05	0.06	-0.31	**-0.69**	1.00					
ECEC	-0.20	0.30	0.06	**0.60**	**0.64**	0.09	-0.18	-0.02	0.10	**0.76**	**-0.58**	**0.99**	**0.40**	**-0.39**	1.00				
Acid-sat	0.24	0.09	**0.38**	0.16	**-0.78**	-0.17	-0.06	-0.30	0.21	-0.08	0.12	**-0.37**	**-0.70**	**0.99**	**-0.45**	1.00			
Zn	-0.22	**0.41**	0.24	**0.61**	0.21	0.09	-0.23	-0.08	0.16	**0.66**	-0.25	**0.57**	-0.11	0.04	**0.53**	0.02	1.00		
Mn	0.16	0.17	**0.37**	0.34	**-0.75**	-0.15	0.19	0.01	-0.10	-0.01	-0.10	-0.17	**-0.67**	**0.74**	-0.27	**0.71**	0.09	1.00	
Cu	-0.34	**0.46**	0.14	**0.83**	0.33	-0.10	-0.16	-0.03	0.10	**0.90**	**-0.59**	**0.94**	0.04	-0.10	**0.90**	-0.16	**0.64**	0.01	1.00

BD: soil bulk density; SOC: soil organic carbon; SOCs: soil organic carbon stocks; Por: porosity; MWD: mean weight diameter; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; EA: exchangeable acidity; Acid sat: acid saturation percentage; Zn: zinc; Mn: manganese; Cu: copper.

3.5 Discussion

3.5.1 Vertical distribution of SOC and SOCs in shrub-encroached and open grassland

This study assessed the vertical distribution of SOC and SOCs within soil profiles of a shrub-encroached grassland compared to adjacent open grassland. The results obtained in this study revealed that SOC was greater in the shrub-encroached grassland than in the open grassland. The increase in SOC in the shrub-encroached grassland can be attributed to the two plant life forms in savanna rangelands (trees and grasses), that control the content, composition and distribution of SOC. These dominant plant life forms differ in their litter chemistry, patterns of detrital input and rooting depth (Chen *et al*., 2005). The increase in cover and abundance of woody plants in savanna rangelands, referred to as shrub encroachment, alters the interactions between trees and grasses, in turn, affecting the level of SOC and SOCs in savanna rangelands (February *et al*., 2020). The change from grass-dominated to woody-dominated vegetation affects the cycling of SOC and nutrients in savanna rangelands (Mogashoa *et al*., 2021). The effect of vegetation cover transformation on SOC has been shown to be associated with changes in SOC input and carbon mineralization (Post and Kwon 2000; Guo and Gifford 2002; Strickland *et al*., 2010; Wessman *et al*., 2012). The higher SOC in the shrub-encroached grassland may be attributed to higher SOC inputs from plant litter fall, mainly dead leaves and roots leading to greater accumulation of SOC. Eldridge *et al*. (2011) reported that shrub encroachment typically results in more biomass carbon input into the upper soil surface, while Jobbágy and Jackson (2000) noted that the quality of SOC input controls the decomposition of the organic material. This study found that SOCs were greater in the open grassland compared to the shrub-encroached grassland. This is because the storage of SOC in soil is governed by the balance of the concentration of SOC and bulk density, with the trade-off between these factors resulting in either an increase or decrease in SOCs (FAO, 2017).

3.5.2 Impact of shrub encroachment on SOC in the topsoil and subsoil layers

The higher SOC (24%) in the topsoil of the shrub-encroached grassland plinthic soil in this study is relatively lower compared to the SOC values reported by other studies elsewhere. For instance, Wang *et al*. (2004) reported that the distribution of SOC in the topsoil layers (0-20 cm) was 46% greater in shrub-encroached grasslands compared to open grasslands, and indicated that soil in the upper 20 cm soil layer stored about 40% of SOC and was more likely to be affected by land-use change and natural disturbance. In contrast, Hudak *et al*. (2003) reported that SOC was less in shrub-encroached grassland compared to open grassland on red clayey loam soils. Jobbágy and Jackson (2000) reported greater SOC in the top 20 cm of grasslands compared to shrub-encroached grassland. The inconsistency of the results from these studies clearly shows that the shift in the cycling of SOC as a result of shrub encroachment depends on the context, which is driven by the interaction between biotic and abiotic (edaphic) factors (Eldridge *et al*., 2011). Moreover, each soil layer within the soil profiles in shrub-encroached grasslands has varying pedogenic processes that will contribute differently to the accumulation of SOC (Ward *et al*., 2018).

This study showed that indeed SOC in the topsoil and subsoil of plinthic soil profiles studied in savanna rangelands is influenced by the different physico-chemical (edaphic) properties as well as the distribution of roots further down the soil profile. The edaphic factors have a profound impact on the migration and transformation of SOC through redistribution of organic matter at different soil layers (Lal, 2004). The effective rooting depth in the soil profiles investigated in this study was up to 30 cm. Similar to Ota *et al*. (2013), his study found that rooting depth influenced the vertical distribution of SOC in the soil profile. Yang *et al*. (2009) observed that about 90% of the roots in grassland areas are concentrated in the top 30 cm of the soil layer, while Jackson *et al*. (1996) found that 83% of the root biomass is found in the first 30 cm of grasslands. When the roots die and decay, they form part of the organic matter, resulting in high microbial activity and better aggregate stability which in turn increases SOC (Rice, 2002). The higher root distribution in the topsoil, contributes to the root-derived stable organic matter, which substantially contributes to most of the SOC currently stored in soils of most ecosystems (Dietzel *et al.,* 2017). The root system is also responsible for the physical protection of SOC in the soil surface and the subsequent storage of SOC in aggregates (Boutton *et al*., 2009; Baumert *et al*., 2018).

In this study, SOCs were greater in the open grassland than in shrub-encroached grassland. The higher SOCs was related to higher BD values measured in the open grassland. The correlation analysis revealed that in both the topsoil and subsoil, BD was negatively correlated with SOC, indicating that a decline in SOC leads to an increase in BD. The increase in BD in the open grassland can be attributed to continuous trampling from grazing animals as it is more accessible than the shrub-encroached grassland, resulting in compacted soil. A compacted soil surface is more likely to limit the grass roots in the deeper soil horizons. Furthermore, compacted soil will significantly reduce the soil infiltration rate and soil water storage. The high BD values observed in the open grassland are also associated with low porosity, which may lead to losses of SOC through surface erosion. Deeper soil layers may hinder the migration of SOC, as they have lower porosity due to the higher BD caused by the overlay of bigger plinthites (Wu *et al*., 2011). Porosity is advantageous to root activity and the coordination of water and organic matter, which helps improve soil structure and SOC storage.

By employing a correlation analysis, this study found that SOC in the subsoil was correlated to various polyvalent cations (calcium, zinc and copper). In savanna rangelands, the availability of polyvalent cations in plinthic soils and their ability to form complexes with organic molecules promotes the chemical protection of SOC (Dlamini *et al.*, 2019). Soil organic carbon in both topsoils and subsoils had a strong positive correlation with P, indicating that an increase in organic matter and subsequent decomposition of soil organic matter leads to more P accumulation. The soil pH of the investigated shrub-encroached and open grassland sites was moderately acidic (3.9–5.6). Under moderately acidic soils, base cations are weakly bound, causing weak interactions with soil organic matter. moderately acidic pH also suppresses microbial biomass leading to low microbial activity, and this consequently affects SOC accumulation (Berthrong *et al*., 2009; Qiu *et al*., 2012).

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3.6 Conclusion

This study quantified and compared the vertical distribution of SOC and SOCs in shrub-encroached and open grasslands on a moderately shallow plinthic soil and also identified the controlling physico-chemical factors. In conclusion, SOC was higher in shrub-encroached grassland in both topsoils and subsoils and greater SOCs in the topsoil were found under open grassland due to higher soil bulk density associated with livestock trampling. In both open and shrub-encroached grasslands, both SOC and SOCs declined with depth. The findings of this study demonstrated that a considerable amount of SOCs is stored in the subsoil of open grasslands when compared to shrub-encroached grassland. Correlation analysis revealed that both SOC and SOCs were chemically and physically stabilized in the subsoil via complexation interactions with polyvalent cations and physical protection by soil aggregates. This has contributed to our understanding that factors controlling SOCs differs in the topsoil and subsoil. The land managers with the responsibility of managing savanna rangelands should therefore consider the vertical variation and highly heterogeneous SOC, SOCs and bulk densities when developing plans aimed at controlling shrub encroachment.

3.7 References

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CHAPTER 5

SUMMARY AND CONCLUSION

The study aimed to investigate the effects of shrub encroachment on the vertical distribution of SOCs and to determine the edaphic factors controlling the level of SOCs in shallow soil profiles of a shrub-encroached grassland. To achieve this objective, the study quantified and compared SOC and SOCs under open and shrub-encroached grassland sited on similar plinthic soil and topographic position. A correlation analysis was run to determine the edaphic factors influencing the distribution of SOC and SOCs in the topsoil and subsoil layers of open and shrub-encroached grassland.

The results obtained in this study revealed that SOC was significantly higher in the shrub-encroached grassland when compared to open grassland, while BD and SOCs were lower in the shrub-encroached grassland relative to open grassland. Overall, SOC, BD and SOCs declined with soil depth in both open and shrub-encroached grassland. The open grassland had greater SOCs due to higher BD, indicating that the open grassland was more compacted compared to shrub-encroached grassland.

The study showed that edaphic factors controlling SOC and SOCs in the topsoil differs to that in the subsoil under both encroachment levels. The correlation analysis revealed that SOC in the topsoil was significantly positively correlated with phosphorus and negatively correlated with BD. In the subsoil, SOC was positively correlated with porosity, phosphorus and polyvalent cations (calcium, zinc and copper) and negatively correlated to BD and mean weight diameter - an indicator of soil aggregate stability. These physico-chemical properties are critical in controlling the accumulation and depletion of SOC in the shrub-encroached plinithic soil profiles investigated in this study.

This study has contributed to a better understanding of the dynamics influencing the vertical distribution of SOCs under open and shrub-encroached grassland soils. The findings of this study not only have demonstrated that deeper soil horizon play a critical role in the storage of SOC in savanna rangelands, but also provides useful baseline data for studying the effects of shrub encroachment on the vertical distribution of SOC in plinthic soils. This study has also provided an improved understanding of the physico-chemical factors controlling SOC in shrub-encroached grassland soils. Accurate quantification of the controlling factors is crucial for improved understanding of the stabilization and destabilization mechanisms of SOC in shrub-encroached grassland soils. Future work may look at the vertical distribution of SOC within micro and macro aggregates, which will further improve our understanding of the physical stabilization mechanisms of SOC.